United States
Environmental Protection Agency

P-15-487-491
Office of Chemical Safety and
Pollution Prevention

Standard Review of P-15-487-491

RAD Dispo Date: 8/12/2015
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SUMMARY TABLE

Property	P-15-0487-491
Chemical (Name, TN,MW, MF, CAS, Str.)	Multi-walled carbon nanotubes MW = 100000; MF = C
Why in Std. Rev.	Carbon nanotubes in standard review for human health concerns
Submitter	Daewoo International USA Corp
Use & PV	Use in electronic devices; 2,000kg/yr
Properties Relevant to Risk (Phys.Form)	Multi-walled carbon nanotubes (MWCNT) properties (range of values for the five PMNs): Number of Walls = 5-20; CNT Diameter = 8-30 nm; CNT Length = 1-20 µm, CNT Aspect Ratio > 200; Bundle Length = 5 - 100 µm; Bundle Aspect Ratio = 0.7 – 200; Wall Ends = Closed
Available Toxicity Data for P-15-0487 only.	1) Acute oral toxicity study in rats 2) Acute dermal toxicity study in rats 3) Acute inhalation toxicity study in rats 4) 28-day inhalation toxicity study in rats 5) 90-day inhalation toxicity study in rats 6) In vitro genetic mutation study in bacteria 7) In vivo bone marrow erythrocyte micronucleus test in mice 8) In vitro mammalian chromosome aberration
Approach Used to Assess Risk	Risk assessment done based on NOAEC of 1.01 mg/m^3 from the 90-day inhalation test using P-15-0487.
Uncertainties	The only 90-day inhalation test data submitted was for P-15-0487; with the shortest bundle length range of 10 – 50 µm. However, potentially longer fibers present in the other PMNs should have higher human health hazard (see Appendix A).
Risk Conclusions	Occupational inhalation risk is supported.
Recommendations	Occupational - PPE: NIOSH-certified particulate respirator with APF of 1000. See Section 6 for testing recommendations.

1 CHEMICAL INFORMATION

1.1 Regulatory History

Submitter: Daewoo International USA Corp
RAD Dispo Date: **8/12/2015**
Day 90: 08/26/2015

Similar Case

[redacted]

1.2 Chemical Identity

(Excerpts from Chemistry Report, 06/11/2015, and Focus Report, 06/19/2015)

PMN differences:

P-15-0487: Multi-walled carbon nanotubes;

P-15-0488: Multi-walled carbon nanotubes;

P-15-0489: Multi-walled carbon nanotubes;

P-15-0490: Multi-walled carbon nanotubes; o

P-15-0491: Multi-walled carbon nanotubes;

Chemical Name:	Multi-walled carbon nanotubes
CAS Number:	None
Mol. Wt.:	100000.00
Mol. Formula:	C
Prod. Vol.	PV Init (kg/yr):400, PV Max (kg/yr):
Mfg. or Import:	The PMN material is imported.
Use:	Additive for electro-static discharge (ESD) in electronic devices, electronics, and materials (additive for weight reduction in materials (additive to improve mechanical properties or electrical conductivities (a heat-generating element in heating devices and materials (additive for heat transfer and thermal emissions in electronic devices and materials (semi-conductor, conductive, or resistive element in electronic circuitry and devices (additive to improve conductivity in electronic circuitry, energy storage systems and devices (electron emitter for lighting and x-ray sources (

additive for electromagnetic interface (EMI) shielding in electronic devices ([redacted] additive for electrodes in electronic materials and electronic devices ([redacted] catalyst support in chemical manufacturing ([redacted] coating additive to improve corrosion resistance or conductive properties ([redacted] additive for fibers in structural and electrical applications ([redacted] additive for fibers in fabrics and textiles ([redacted] filter additive to remove nanoscale materials ([redacted] semi-conducting compounding additive for high-voltage cable (3%), and additive for super-hydrophobicity ([redacted] Consoildated Set: P-15-487, P-15-488, P-15-489, P-15-490, and P-15-491.

Physical States: Solid

Mfg/Import: Imported

Other Use: **Submitted PMN could be used also as:**

[redacted]

[redacted]

SAT Rating: Health 2, Eco 1
P B T Call: NANO

The molecular weight is variable and depends on the size of the nanotube. It is estimated to be >1,000,000 g/mole. The combined production volume for the consolidated set is INIT: [redacted] kg/yr; MAX: [redacted] kg/yr. Submitted Properties: Agglomerates as bundles; Number of Walls = 5-20; CNT Diameter = 8-30 nm; CNT Length = 1-20 µm, CNT Aspect Ratio > 200; Bundle Length = 5-100 µm; Bundle Aspect Ratio = 0.7-200; Wall Ends = Closed; Purity > 93%; Catalyst Particle Size = 1-150 µm; WS = Insoluble; Density = 0.015-0.030 g/cc (Powder), 0.060-0.140 g/cc (Pellet); Particle Size (6 runs): D10 = 10.00-11.56 µm, D50 = 33.35-39.52 µm, D90 = 101.97-134.54 µm. Estimated Properties: VP < 0.000001 torr (High MW); WS < 0.000001 g/L (High MW).

1.3 Structure and Composition

(Per Chemistry Report, 06/11/2015)

Figure 1 Chemical Structure

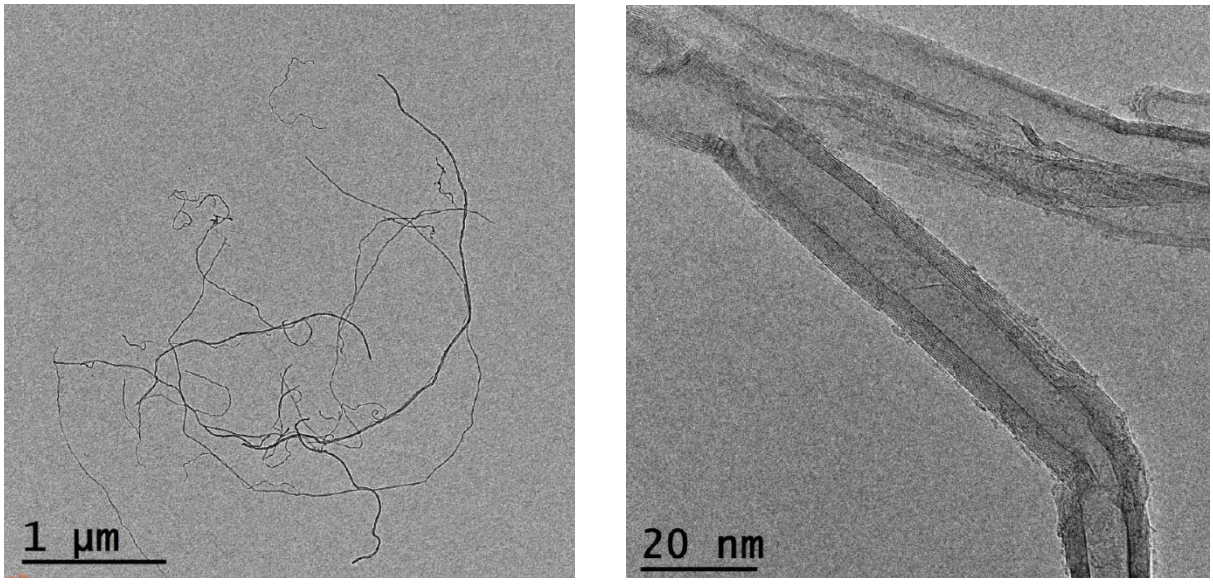

Figure 2 A-TEM Images for PMN

1.4 Fate Information

Fate Summary Statement
(Excerpts of Fate Summary from Focus Report, 06/11/2015).
M=Measured; E=Estimated
Solid
S < 0.001 mg/L at 25 °C (E)
VP < 1.0E-6 torr at 25 °C (E)
BP > 400 °C (E)
H < 1.00E-8 (E)
POTW removal (%) = 0-90 via possible sorption
Time for complete ultimate aerobic biodeg > mo
Sorption to soils/sediments = low - v.strong
PBT Potential: NANO
FATE: Migration to ground water = negl - rapid

1.5 Manufacturing Process

(Per Post Focus Chemistry Report, 06/11/2015)
The PMN material is imported, and only a very brief manufacturing process diagram was provided. The PMN material is manufactured [redacted]

1.6 Test Data Submitted

Test data for P-15-0487:

In an acute oral toxicity study, a group of three female Sprague-Dawley (SD) rats were administered P-15-0487 (purity: >90%) in DPPC solution via gavage at 300 mg/kg bw and observed for 14 days. No mortality or clinical signs of toxicity were observed within 6 days in the first group of rats, so a second group of three female rats received the same dose and were observed for 14 days. No mortality or clinical signs of toxicity were noted. Body weight gain was normal in all animals throughout the study period. Necropsy revealed no macroscopic abnormalities. The acute oral LD50 was > 300 mg/kg-bw in female rats.

In an acute dermal toxicity study, Sprague-Dawley CD rats (5/sex/group) were dermally exposed to a single dose of P-15-0487 (purity: > 90%) at 0 or 2000 mg/kg-bw and observed for 14 days. P-15-0487, moistened with DPPC solution, was applied to the clipped, intact skin of each rat and held in place for 24 hours under semi-occlusive dressing. Following the exposure period, the dressings were removed and the test site was washed with sterile distilled water. No mortalities occurred and no signs of toxicity or skin irritation were noted during the study. No treatment-related effects on body weight were observed. Necropsy revealed no macroscopic abnormalities. The acute dermal LD50 was > 2000 mg/kg-bw in male and female rats.

In an acute inhalation toxicity study, Fisher 344 rats (5/sex/group) were exposed whole-body to P-15-0487 (purity: > 90%) for 6 hours at measured concentrations of 0 (filtered fresh air), 0.17, 0.52, and 0.83 mg/m^3 and were observed for 14 days post-exposure. No treatment-related mortality or signs of toxicity were observed during the study. There were no significant effects on body weight. No treatment-related effects were observed at necropsy. The 6-hour LC50 was > 0.83 mg/m^3 in male and female rats.

In a 28-day repeated-dose inhalation toxicity study, Fischer 344 rats (10/sex/group) were exposed, nose-only, to P-15-0487 (purity: > 90%) at measured concentrations of 0, 0.17, 0.51, and 0.97 mg/m^3 for 6 hours/day, 5 days/week, for 28 days. No mortalities or PMN substance-related adverse effects were observed. The NOAEC for male and female rats was > 0.97 mg/m^3.

In a 90-day repeated-dose toxicity inhalation study, Fischer 344 rats (10/sex/group) were exposed, nose-only, to P-15-0487 (purity: > 90%) at measured concentrations of 0 (filtered fresh air), 0.17, 0.51, and 1.01 mg/m^3 for 6 hours/day, 5 days/week, for 13 weeks. Additional male rats (5/group) were included in the control, low-, mid-, and high-concentration groups for the assessment of recovery after a 13-week non-exposure period. No PMN substance-related mortalities or adverse effects were observed. The NOAEC for male and female rats was > 1.01 mg/m^3.

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed to P-15-0487 (purity: > 90%) in DPPC solution at concentrations ranging from 31 to 500 µg/plate, with and without metabolic activation. Vehicle and positive controls were included and responded appropriately. No evidence of cytotoxicity was observed. No information was provided regarding test substance precipitation. No increase in revertants was observed at any concentration with or without metabolic activation.

In an in vivo micronucleus assay, ICR mice (6 males/group) were exposed to P-15-0487 (purity: 90%) in DPPC solution at concentrations of 12.5, 25 or 50 mg/kg-bw. No signs of systemic toxicity or cytotoxicity were observed. Negative (vehicle) and positive controls were included and responded appropriately. The test item did not induce micronuclei in male or female mice exposed to P-15-0487 under the conditions of this study.

Chinese hamster ovary (CHO-k1) cells were exposed to P-15-0487 (purity: > 90%) in DPPC solution at concentrations ranging from 0.78 to 3.13 µg/mL, with and without metabolic activation. Negative (vehicle) and positive controls were included and responded appropriately. In a preliminary range-finding test, cytotoxicity was observed at concentrations ≥ 3.13 µg/mL in the presence and absence of metabolic activation, and test substance precipitation was observed at concentrations ≥ 6.25 µg/mL. No information regarding cytotoxicity or test substance precipitation was reported in the main study. No increase in the number of aberrant metaphases was observed at any concentration with or without metabolic activation.

1.7 Focus Decision and Rationale (Excerpts from FOCUS report from 06/22/2015)

P-15-0487, P-15-0488, P-15-0489, P-15-0490, P-15-0491 will be placed into a standard review for human health and ecotoxicity concerns. A full team will be needed for the review process. Human health hazard concerns were moderate for dermal and inhalation exposures. The SAT reviewers found this chemical to fit the human health category of respirable poorly soluble particulates. Ecotoxicity concerns were low. Potential risks to workers and the environment will be determined during the review process.

2 TOXICITY / HAZARD SUMMARIES

2.1 Human Health Summary

2.1.1 Absorption/Metabolism

Poor systemic absorption all routes for the fraction < 100 nm based on physical/chemical properties; however, for nanomaterials portal entry are the concern.

2.1.2 Human Health Effects of Concern

There is a concern for adverse lung effects (i.e., lung overload and lung cancer) if poorly soluble respirable particulates and fibers are inhaled. There is uncertain concern for mutagenicity and immunotoxicity for nano materials.

Cancer Concern:
Per Standard Review for close analog [redacted] - Appendix A.
By analogy to [redacted] and based on data for some carbon nanaotubes (MWCNT and SWCNT, single-walled carbon nanotubes), there is a concern of the PMN for pulmonary toxicity, fibrosis and cancer. There is evidence that some carbon nanotubes can translocate from the respiratory tract to the regional lymph node and can cause pleural inflammation in mice. Therefore, there is also concern for pleural toxicity, fibrosis and cancer (mesothelioma) if inhaled. In addition, there are data suggesting that pulmonary deposition of some nanoparticles, including carbon nanotubes, may induce cardiovascular toxicity when inhaled.

Immunotoxicity:
Per Standard Review for close analog [redacted] - Appendix B.
Respirable particles are an immunotoxicity hazard; potential to cause adverse effects to the lung, including inflammation, cell damage, reduced bacterial clearance, and systemic immunotoxicity.

2.1.3 Human Health Toxicity - Toxicity data for P-15-0487.

The toxicity summary (below) of the 90-day inhalation study for P-15-0487 is used to assess risk. Study review done reviewed by SRC with RAD Quality Assurance is in the attached file (Appendix C) and the submitted toxicity studies are available in CIS at [redacted]

90-day inhalation toxicity study in rats
Title: Subchronic Inhalation Toxicity Study of MWCNT in Fischer 344

Choi, B-G. 2014c. Subchronic Inhalation Toxicity Study of MWCNT in Fischer 344. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea. Study No. GT14-00042. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; black powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fisher 344 rats (10/sex/group) were exposed nose-only to P-15-0487 for 6 hours/day, 5 days/week, for 13 weeks at target concentrations of 0 (filtered fresh air), 0.2, 0.5, or 1 mg/m^3 (analytically measured concentrations: 0, 0.17, 0.51, and 1.01 mg/m^3, respectively) of dry substance in the air. Additional male rats (5/group) were included at all concentrations for the assessment of recovery after a 13-week non-exposure period. Target exposure concentrations were selected based on the results of an acute inhalation toxicity study in rats and a 28-day repeated-dose inhalation study in rats (Choi, 2014a, b; reviewed herein) in which no mortality or toxic signs were observed at the highest concentrations tested, 0.83 mg/m^3 and 0.97 mg/m^3, respectively. The highest exposure concentration for this study was the maximal mass concentration capacity for the carbon nanotube generating system. The geometric mean cumulative median length (±SD) of the PMN substance was 566.54±1.88 nm. Continuous data were analyzed using the standard one-way analysis of variance and Duncan's or Dunnett's test. Non-continuous data were analyzed by Chi-squared analysis. Statistical analyses were conducted with a significance level of $p < 0.05$. The study, which was conducted in 2014, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-2 (2013) and OECD TG 413 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The NOAEC for male and female rats is > 1.01 mg/m^3, based on no adverse treatment-related effects observed at the highest concentration tested. A LOAEC could not be determined.

One animal in the mid-concentration group died on study day 81 after exhibiting restlessness, convulsions, and stupor on study day 80. The animal had red exudate in the abdominal cavity; inflammation, flattening of the uroepithelium, lumen dilatation, and red urine in urinary bladder; pulmonary and hepatic congestion; focal mineralization of the renal tubule; and prostate edema, hemorrhage, and inflammation. The death was not considered to be related to exposure.

No other mortalities or clinical signs were observed in the main study or during the recovery period. In the main study, there were no PMN substance-related effects on body weight, food consumption, ophthalmoscopy, hematology and blood coagulating parameters, female urinalysis parameters, female absolute organ weights, relative organ weights, gross necropsy findings, bronchoalveolar lavage test, or microscopic findings. During the recovery period, there were no differences in body weight, food consumption, ophthalmoscopy, hematology parameters, urinalysis parameters, absolute and relative organ weights, gross necropsy findings, bronchoalveolar lavage test, or microscopic findings among the groups.

In female rats of the main study, significantly increased ($p<0.05$) sodium levels (2%) were observed at all concentrations and significantly increased potassium (6%) levels were observed at the mid- and high-concentrations, compared with controls. During the recovery period, prothrombin time was significantly ($p<0.05$) increased by 10 and 9%, in males of the mid- and high-concentration groups, respectively. In males of the low- and high-concentration recovery groups, cholesterol levels were significantly increased by 8 and 7%, respectively, and magnesium levels were significantly decreased by 16 and 13%, respectively. In the main study, there was a statistically significant increase ($p<0.05$) in trace urine ketone bodies in males of the mid- and high-concentration groups; incidence of 0/5, 1/5, 3/5, and 5/5 at control, low-, mid-, and high-concentration, respectively. The incidence of urine ketone bodies (grade 1+) was 5/5, 4/5, 1/5, and 0/5 at control, low-, mid-, and high-concentration, respectively, showing a concentration-related decreasing trend with statistical significance in the mid- and high-concentration groups. A concentration-related trend for increasing urine pH values in PMN substance-exposed males was also observed. The findings were of uncertain toxicological relevance due to lack of gross and histological findings in the kidney. A statistically significant increase (18%) in absolute left lung weight was observed in males of the high-concentration group. The study author stated that the significant blood coagulation, clinical chemistry, and organ weight changes were not treatment-related as the values either fell within the normal physiological range and/or the changes were not concentration-dependent. No data demonstrating the normal physiological range were included in the study report. However, the biological relevance of the blood coagulation, clinical chemistry, and urine ketone body and pH changes is unknown as there were no correlating changes in organ weights or histopathology. Histopathological changes were observed in one or two animals or at a similar incidence in the control and exposed males and were not considered to be PMN substance-related.

Conclusions:
Author's conclusions: The NOAEC for male and female rats is > 1.01 mg/L, and a target organ was not identified.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion that the NOAEC for male and female rats is > 1.01 mg/L, based on no adverse treatment-related effects observed at the highest concentration tested; the LOAEC could not be determined. It is noted that the highest concentration tested did not result in toxic effects, but the highest concentration was the maximal mass concentration capacity for the carbon nanotube generating system. It is also noted that no females were included in the recovery group, and justification was not provided.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

2.2 Environmental (Aquatic) Hazard Summary

RAD is currently evaluating available information on the aquatic toxicity hazard of nanomaterials. As this review continues, a policy decision has been made to identify the hazard as "high" based on this uncertainty.

2.2.1 Concentrations of Concern (CoC)

The chronic COC is derived by using the worst-case hazard scenario and assigning a value of 1 ppb.
Chronic CoC = 1 ppb

3 RELEASES AND EXPOSURES

3.1 Environmental Releases and Exposures

3.1.1 Occupational Exposures

(Per Engineering Report, 06/12/2015)

Table 1 Workers can be exposed to the PMN substance during the following scenarios and activities:

a

Scenario		**# Sites**	**# Workers**
Use 1: Incorporation as Additive in Articles (PV)		[redacted]	[redacted]
Exposure		**mg/day**	**day/yr**
Inhalation - Particulate	Worst Case / Typical	[redacted]	[redacted]
Dermal - Solid	High End	[redacted]	[redacted]
Release		**kg/site/day**	**day/yr**
Water or Air or Incineration or Landfill	Conservative	[redacted]	[redacted]
Water or Incineration or Landfill	Output 2	[redacted]	[redacted]
Water or Incineration or Landfill	Output 2	[redacted]	[redacted]
Water or Incineration or Landfill	Output 2	[redacted]	[redacted]

b

Scenario		# Sites	# Workers
Proc 2: Formulation of Coatings Additive (PV))			
Exposure		**mg/day**	**day/yr**
Inhalation - Particulate	Worst Case / Typical		
Dermal - Solid	High End		
Dermal - Liquid	High End		
Release		**kg/site/day**	**day/yr**
Water or Air or Incineration or Landfill	Conservative		
Water or Incineration or Landfill	Output 2		
Water or Incineration or Landfill	Output 2		

c

Scenario		# Sites	# Workers
Use 2: Application of Coatings (PV)			
Exposure		**mg/day**	**day/yr**
Inhalation - Mist	Upper Bound		
Dermal - Liquid	High End		
Release		**kg/site/day**	**day/yr**
Water or Incineration or Landfill	Conservative		
Air	Output 2		
Landfill	Output 2		

3.1.2 General Population Exposures

(Per Exposure Report, 06/19/2015)

Table 2 Predicted exposure estimates for P-15-487-491: Dose, Concentration, and Days Exceeded Results.

Exposure Scenario[1]	Water						Landfill	Stack Air		Fugitive Air	
Release activity(ies)[2]; exposure calculation(s)[3]	Drinking Water		Fish Ingestion		7Q10[4] CoC = 1	PDM Days Exceeded	LADD	ADR	LADD	ADR	LADD
	ADR	LADD	ADR	LADD							
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	µg/l	# Days	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
PROC 2: Max ADR: max acute eco	**2.11E-04**	---	**1.09E-02**	---	**1.04E+01**	[redacted]	---	**6.04E-06**	---	**1.75E-05**	---
PROC 2: PDM1	---	---	---	---	**1.04E+01**		---	---	---	---	---
PROC 2: PDM2	---	---	---	---	**4.47E+00**		---	---	---	---	---
PROC 2: Max LADD	---	**1.28E-06**	---	**4.63E-05**	---		**2.78E-07**	---	**1.90E-07**	---	**4.06E-07**
USE 1: Max ADR: max acute eco	**2.15E-04**	---	**1.11E-02**	---	**1.06E+01**		---	**6.15E-06**	---	**1.98E-05**	---
USE 1: PDM1	---	---	---	---	**1.06E+01**		---	---	---	---	---
USE 1: PDM2	---	---	---	---	**7.21E+00**		---	---	---	---	---
USE 1: Max LADD	---	**1.79E-06**	---	**6.45E-05**	---		**3.86E-07**	---	**2.65E-07**	---	**4.59E-07**
USE 2: Max ADR: max acute eco	**1.81E-04**	---	**9.38E-03**	---	**8.94E+00**		---	**5.18E-06**	---	**2.75E-04**	---
USE 2: PDM1	---	---	---	---	**8.94E+00**		---	---	---	---	---
USE 2: Max LADD	---	**2.03E-06**	---	**7.30E-05**	---		**1.03E-05**	---	**3.00E-07**	---	**6.37E-06**

[1] Exposure scenario titles consist of release activity followed by exposure calculation abbreviation.

[2] Release activities are from engineering report's Manufacturing (Mfg), Processing (Proc) and Use release activity labels. Multiple release activities are combined in one exposure scenario if their releases occur at same location.

[3] Exposure calculations are Acute Dose Rate (ADR), Lifetime Average Daily Dose (LADD), and Probabilistic Dilution Model (PDM). There may be one, two, or all three exposure calculations per exposure scenario. CC is the aquatic concentration of concern.

[4] This column displays concentration values for the 7Q10 streamflow, which is defined as the average daily streamflow of the seven consecutive days of lowest flow within a ten year period.

3.1.3 Consumer Exposures

(Per Exposure Report, 06/19/2015)

There are no predicted exposures resulting from consumer use of the PMN substance.

3.1.4 Exceedences in Surface Water

The following exposure release scenarios were estimated for PMNs P-15-0487/0488/0489/0490/0491. EPA's probabilistic dilution model (PDM) provide predicted surface water concentrations based on the specifications given by the submitter and the number of days the 7Q10 surface water concentration is expected to exceed the chronic COC. The 7Q10 is the lowest stream flow for seven consecutive days that would be expected to occur once in 10 years. Significant chronic risk occurs if the surface water concentration exceeds the chronic COC by twenty days or more. The maximum acute ecological (max acute eco) designation describes the maximum surface water concentration or 7Q10 value with a given scenario for the PMN material. It is typical for the exposure assessment team to assess CNT materials using a worst-case scenario, as the input parameters to the model, due to lack of information regarding removal rates and surface water concentrations using a chronic COC of 1 ppb. The exposure releases from the exposure reports are shown below:

Chronic COC = 1 ppb for PMNs P-15-0487/0488/0489/0490/0491

Exposure Scenario	Release Activity	***7Q10 (µg/L)	Days Exceeded
Processing 2	max acute eco (*ADR)	11	-
Processing 2	**PDM1	10	[redacted]
Processing 2	PDM2	4.7	[redacted]
Use 1	max acute eco (ADR)	10	-
Use 1	PDM1	10	[redacted]
Use 1	PDM2	7.2	[redacted]
Use 2	max acute eco (ADR)	8.9	
Use 2	PDM2	8.9	[redacted]

*ADR =Acute Dose Rate;
**PDM =Probabilistic Dilution Model;
***7Q10 = the lowest stream flow for seven consecutive days that would be expected to occur once in 10 years

4 RISK ASSESSMENT

4.1 Human Health Risk Discussion

4.1.1 Effects Levels Used to Determine Risk

The inhalation toxicity NOAEC of 1.01 mg/m^3 from the 90-day inhalation study submitted for P-15-0487 is used to assess risk. Study details are in sections 1.6 and 2.1.3.

4.1.2 Occupational Risk Discussion

As shown in the table below, when the occupational inhalation doses are compared to the inhalation toxicity with NOAEC of 1.01 mg/m^3 from the 90-day inhalation test using the PMN P-15-0487 unacceptable MOE = 0.264 (<100) are estimated for inhalation exposure during the use 2: Application of

Coatings. Other two predicted scenarios: Incorporation as Additive in Articles (Use 1) and Formulation of Coatings Additive (Processing 1) - resulted in acceptable MOE: 158.6 and 172.3, respectively.

Table 3 Occupational Exposure MOE Calculations for P-15-0487

Occupational Exposure MOE Calculations for P-15-0487-0491 using the NOAEC 1.01 mg/m3 from 90-day Inhalation Study for the analog P-15-0487.

Exposure Scenarios and Values	Potential Dose Rate (mg/day)	PMN Inhalation Exposure (mg/m^3)*	NOAEC, 90-d inhal for P15-487 (mg/m^3)	NOAEC from 6hr to 8hr exp (mg/m^3)	NOAEC ajucted active/rest (mg/m^3)**	Margin of Exposure (NOAEC/ PMN Dose)	Inhalation "Fold" Factor^^ (100/MOE)
Use 1: Incorporation as Additive in Articles [redacted] PV), [redacted] sites, [redacted] d/yr, [redacted] workers							
Inhalation-Particulate-Worst Case [2.5E-2]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Proc 2: Formulation of Coatings Additive [redacted] PV), 4 sites, [redacted] d/yr, [redacted] workers							
Inhalation-Particulate-Worst Case [2.3E-2]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
USE 2: Application of Coatings , [redacted] sites, [redacted] d/yr, [redacted] workers							
Inhalation-Mist-Upper Bound [1.5E+1]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

*Occupational inhalation exposures are from the engineering report (06/12/2015) and are calculated as follows (the reported exposures are 15 mg/day):
15 mg/d divided by 10 cubic meters of air (the amount of air breathed by workers over an eight hour work day) = 1.5 mg/m^3.
**NOAEC adjusted to 8 hours exposure and for active/rest breathing rate (example with 1.01): 1.01 x 6/8 x 15.7/30 = 0.3964 mg/m^3.
^^Fold factor = value to be applied to bring INHALATION MOE up to acceptable level, used by the CEB Industrial Hygienist to determine respirator recommendations. NOAEC/NOAEL-based fold factor = 100/MOE; LOAEC/LOAEL-based fold factor = 1000/MOE
An acceptable MOE for a NOAEL-based assessment is ≥100 and for a LOAEL-based assessment it is ≥1,000.

4.1.3 General Population Risk Discussion

When the general population estimated Max 24 Hour Average Air Concentration and Max Annual Average Air Concentration expose doses to Fugitive or Stack Air are compared to the inhalation toxicity with NOAEC of 1.01 mg/m^3 from the 90-day inhalation test using the PMN P-15-487 acceptable MOE (>100) are estimated for inhalation exposure during the all scenario, suggesting no unreasonable calculated risk of injury.

4.2 Environmental Risk Discussion

Ecological risk was evaluated for P-15-0487/0488/0489/0490 /0491 using a conservative prediction for high ecological hazard (chronic COC of 1 ppb) with exposure scenarios assuming 0% degradation in POTWs. High chronic ecological risks were determined for P-15-0487/0488/0489/0490/0491 from the predicted release scenarios during both use 1 and use 2. The 7Q10 surface water concentrations exceeded the chronic COC of 1 ppb

up to 35 days per year, the regulatory/policy determination for chronic risk occurs when the surface water concentration exceeds the chronic COC for 20 days or more per year.

5 CONCLUSIONS

5.1 Human Health Conclusions

5.1.1 Occupational Health Conclusions

Occupational inhalation risks are predicted for unprotected workers (estimated to be [redacted] total workers for [redacted] days per year at [redacted] sites) who use PMN during Use 2: Application of Coatings.

5.1.2 General Population Conclusions

General population calculated risk is not supported from inhalation of released Fugitive or Stack.

5.2 Environmental / Aquatic Conclusions

Ecological risk was evaluated for P-15-0487/0488/0489/0490 /0491 using a conservative prediction for high ecological hazard (chronic COC of 1 ppb) with exposure scenarios assuming 0% degradation in POTWs. High chronic ecological risks were determined for P-15-0487/0488/0489/0490/0491 from the predicted release scenarios during both use 1 and use 2. The 7Q10 surface water concentrations exceeded the chronic COC of 1 ppb up to 35 days per year, the regulatory/policy determination for chronic risk occurs when the surface water concentration exceeds the chronic COC for 20 days or more per year.

6 RECOMMENDATIONS

6.1 Human Health Recommendations

6.1.1 Occupational Health Recommendations

Based on the results from the 90-day inhalation test using PMN P-15-0487 with NOAEC of 1.01 mg/m^3, occupational inhalation risks are predicted. MSDS should state: “Toxic if inhaled”. Calculated “Fold factor” (times of exposure reduction to acceptable level) for [redacted] workers at [redacted] sites was [redacted] suggesting use of NIOSH-certified particulate respirator with APF of 1000.

Table 4 Company MSDS extraction (Per Engineering Report in PMN GOLD, 3/30/2015)

MSDS:	Yes	Label:	No
General Equipment:	A system of local and/or general exhaust is recommended to keep employee exposures above the Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. The use of local exhaust ventilation is recommended to control emissions near the source. // Wear primary eye protection such as splash resistant safety goggles with a secondary protection face shield. /// Wear appropriate gloves. /// Wear appropriate clothes		
Respirator:	Under conditions of frequent use or heavy exposure, respiratory protection may be needed. Respiratory protection is ranked in order from minimum to maximum. Consider warning properties before use. (1) Dust, mist, fume-purifying respiratory protection: any air-purifying respirator with a corpuscle filter of high efficiency; any respiratory protection with an electromotion fan (for dust, mist, fume-purifying) high-efficiency particulate filter respirator attached self-service protector. (2) For unknown concentration or immediately dangerous to life or health: supplied-air respirator (hybrid air-line mask) or supplied-air respirator with full facepiece.		
Health Effects:	Causes serious eye irritation. May cause respiratory tract irritation.		
TLV/PEL (PMN or raw material):	- Synthetic Graphite - >94% - TWA - ACGIH (TLV)		

6.1.2 Testing Recommendations.

Considering very little knowledge of long term effect of MWCNT and cancer concern for this class of chemicals Combined Chronic Toxicity/Carcinogenicity Test (OPPTS 870.4300) via inhalation route. P-15-0491 suggested if only one substance will be tested (see Appendix A for justification).

This scenario results in a significant risk for chronic ecotoxicity and the chronic base-set (i.e., fish, daphnia, and green algae) is recommended. Refer to summary table below. Also, protocol reviews are required prior to conducting the tests. Not all PMN's need to be tested.

Species	**EPA Test Guidance**	**other parameters**
Fish Early Life-Stage	OCSPP 850.1400	Flow-through method, analytical measurement of test substance
Chronic Daphnia	OCSPP 850.1300	Flow-through method, analytical measurement of test substance
Algae	OCSPP 850.4500	Analytical measurement of test substance

Appendix A

May 1, 2009

MEMORANDUM

SUBJECT: Health Hazard Review of [redacted]

FROM: David Lai (ECAB/RAD)

TO: Sylvon Vonderpool (NCSAB/RAD)

THRU: Jennifer Seed (ECAB/RAD)

Attached is my review of the health hazard potential of the subject PMN. Please contact me should you have any questions regarding this review.

This memo contains [redacted]

Attachment
cc:
Bob Morcock

Health Hazard Review of [redacted]

I. CONCLUSION

[redacted] are multiwalled carbon nanotubes (MWCNT) with an average diameters of 11-12 nm (6-8 walls) and an average length of 5-10 µm. The PMN substance exists as simple and complex agglomerates of carbon nanotubes by weaving around each other by van der Waals force. The carbon nanotube agglomerates are rough spherical or irregular in shape and their dimension ranges from about 10-100 µm. Some of the small agglomerates (≤10 µm) are respirable in humans. Furthermore, the agglomeration strength of these agglomerates is not very high and they are considered loose agglomerates having a lot of empty space between simple agglomerates and carbon nanotubes. Once deposited in the alveolar region, simple aggregates of [redacted] may de-agglomerate to carbon nanotubes when interacted with lung surfactant and some of the carbon nanotubes may exhibit their effects as asbestos fibers. Therefore, by analogy to asbestos fibers and based on data of some carbon nanaotubes (MWCNT and SWCNT, single-walled carbon nanotubes), there is concern of the PMN for pulmonary toxicity, fibrosis and cancer. There is evidence that some carbon nanotubes can translocate from the respiratory tract to the regional lymph node and can penetrate the pleura to cause pleural inflammation in mice. Therefore, there is also concern of the PMNs for pleural toxicity, fibrosis and cancer (mesothelioma) if inhaled. In addition, there are data suggesting that pulmonary deposition of some nanoparticles, including carbon nanotubes, may induce cardiovascular toxicity when inhaled.

II. BASIS OF CONCLUSION

[redacted] are multiwalled carbon nanotubes (MWCNT) with an average diameters of 11-12 nm (6-8 walls) and an average length of 5-10 µm. The PMN substance exists as simple and complex agglomerates of carbon nanotubes by weaving around each other by van der Waals force. The carbon nanotube agglomerates are rough spherical or irregular in shape and their dimension ranges from about 10-100 µm (size distribution not provided). Some of the small agglomerates ($\leq$10 µm) are respirable in humans. The agglomeration strength of these agglomerates is not very high and they are considered loose agglomerates having a lot of empty space between simple agglomerates and carbon nanotubes (PMN submission). Dispersion of SWCNT has been demonstrated using natural lung surfactant and the dispersed form of SWCNT was more cytotoxic and fibrotic than the non-dispersed form (Wang et al., 2009). Once deposited in the alveolar region, simple aggregates of [redacted] may de-agglomerate to carbon nanotubes when interacted with lung surfactant and some of the carbon nanotubes may exhibit their effects (through oxidative stress and inflammation) as asbestos fibers (Donaldson et al., 2006). A recent panel review has identified many similarities between high aspect ratio nanoparticles (HARN) and asbestos with regard to their physicochemical properties and toxicological effects and has concluded that there is sufficient evidence to suggest that HARN which have the same characteristics (diameter, length and biopersistence) as pathogenic fibers are likely to have similar pathology (Tran et al., 2008).

Asbestos fibers are well-documented human carcinogens (EPA, 1993). Although the mechanisms involved in fiber carcinogenesis are not clearly understood, there appears to be a general belief that fiber dimension and tissue burden, which is determined by rates of deposition and clearance, are of primary importance. Experimental evidence accumulated from studies of asbestos and other mineral fibers over the last three decades has shown that long, thin (thus high aspect ratio) fibers are more carcinogenic than short and thicker fibers. In humans, fibers having a diameter of approximately 3.5 µm or less are respirable and are readily deposited in the lung by sedimentation. Thin fibers with a length up to 200 µm may be able to travel to distal segment of the lung and are deposited in the alveoli. Since long fibers are only partially engulfed by macrophages, they are less likely to be cleared out of the alveolar compartment and may remain in the lungs for a longer period of time to interact with epithelial cells. Hence, both the aerodynamic consideration of fiber deposition and the clearance mechanism of the fibers in the respiratory tract are consistent with the Stanton hypothesis that long, thin fibers are more carcinogenic than short and thicker fibers (Stanton et al., 1977). Whereas direct genotoxic effects of asbestos and several man-made fibers have been reported when tested in some *in vitro* assay systems, inhalation studies with animals have shown a strong association of chronic inflammation with the development of fibrosis and cancer in the lungs and pointed toward an indirect mechanism which involved activation and persistent inflammation of alveolar macrophages and epithelial cells with the release of cytokines, growth factors and ROS, leading to DNA damages, cell proliferation, fibrosis, and finally tumor formation.

There are several *in vitro* and *in vivo* toxicity studies on single-walled carbon nanotubes (SWCNT) suggesting that they may have various toxic properties. For instance, a cytotoxicity test was conducted in alveolar macrophage (AM) for SWCNT; quartz (crystalline silica) was used as a positive control (Jia et al., 2005). The SWCNT tested have a mean diameter of about 1.4 nm and a mean length of about 1 µm. The surface area of SWCNT varies with the degree of aggregation/agglomeration of the material since no dispersing reagent was used. Trace amounts of the catalysts Fe, Y and Ni remained as

impurities. The cytotoxicity induced by the tested materials was determined by the MTT assay. Profound cytotoxicity of SWCNT was observed in alveolar macrophages (AM) after a 6-h exposure *in vitro*. The AM showed characteristic features of necrosis, degeneration and a sign of apoptotic cell death. It also significantly impaired phagocytosis of AM. The cytotoxic response of SWCNT was also demonstrated in human dermal fibroblasts in culture; sidewall functionalized SWCNT [*e.g*., with pheny-SO_3H, phenyl-$(COOH)_2$] were found to be less cytotoxic (Sayes et al., 2005).

Shvedova et al. (2005) have demonstrated that pharyngeal aspiration of SWCNT (diameter,1-4 nm; surface area,1,040 m^2/g; length unspecified) induces a robust acute inflammation reaction with very early onset of a fibrotic response and the formation granulomas in mice. The findings appear to suggest a new mechanism for the toxicity of nanomaterials as the mechanism for the early onset yet progressive fibrosis in the absence of persistent inflammation differs from mechanisms proposed for classical fibrogenic particles in that it is not driven by chronic inflammation. Similarly, studies conducted by Lam et al. (2004) and Warheit et al.(2004) examining the pulmonary toxicity of SWCNT by intratracheal instillation in mice and rats, respectively, have both reported an epithelial granulomatous reaction to SWCNT.

The respiratory toxicity of a multi-walled carbon nanotube (MWNCT) has been studied in rats by intratracheal instillation (Muller et al., 2005). Intact MWNCT (15 carbon layers on average, average outer diameter: 9.7 nm; average length: 5.9 µm, specific surface area: 378 m^2/g) and ground MWNCT (15 carbon layers on average, average outer diameter: 11.3 nm; average length: 0.7 µm, specific surface area: 307 m^2/g) were administered intratracheally (0.5, 2, or 5 mg/animal) to Sprague-Dawley rats and biopersistence, inflammation and fibrosis were assessed on days 3, 15, 28 and 60. After 60 days, significant amounts (80% and 40% of the lowest dose) of both intact and ground MWNCT were still present in the lungs and both induced inflammation and fibrotic reactions. Furthermore, the pro-inflammatory and pro-fibrotic mediator TNF-α was up-regulated in response to the carbon nanotubes in vivo and in vitro. The data suggested that the MWNCT are biopersistent and are capable of stimulating lung cells to produce TNF-α to induce lung inflammation, fibrosis, and probably tumors. Exposures to MWCNT by pharyngeal aspiration (Wolfarth et al., 2009) or by short-term (up to 8 days) or subchronic (90-days) inhalation (Landsiedel et al., 2009; Porter et al., 2009) have resulted in dose-dependent increases in pulmonary inflammation and evidence of fibrosis in rats and mice. The LOAEC of the 90-day inhalation study in rats is 0.1 mg/m^3 ((Landsiedel et al., 2009).

Inhaled particles in the nanosize range can certainly deposit in all parts of the respiratory tract including the alveolar region of the lungs. Because of their small size, they may pass into cells directly through the cell membrane and distribute throughout the body once translocated to the blood circulation. There is evidence that nanoparticles can translocate from the portal of entry, the respiratory tract, via different pathways to other organ/tissues. Therefore, with respect to nanoparticles, there is concern for systemic effects (target organs, cardiovascular, neurological toxicities, etc.) in addition to portal-of-entry (e.g., lung, skin, intestine) toxicity.

Exposing the mesothelial lining of the body cavity of mice (as a surrogate for the mesothelial lining of the chest cavity) to long MWCNT by injection has resulted in asbestos-like, length-dependent, pathogenic behavior (Poland et al., 2008). Evidence that MWCNT can cause persistent pulmonary inflammation, can be translocated from the lung to the regional lymph node and can penetrate the pleura to cause pleural inflammation has indeed been shown in mice exposed by pharyngeal aspiration

(Hubbs, et al., 2009) or by inhalation (Bonner et al., 2009).

Pulmonary deposition of styrene nanoparticles was found to not only elicit pulmonary inflammation but induce vascular thrombosis (Nemmar et al., 2003). Pulmonary deposition of carbon black nanoparticles was found to decrease heart rate variability in rats and prolong cardiac repolarization in young healthy individuals in a recent clinical study (Holker et al., 2005). Various cardiovascular dysfunctions have been reported in rats exposed to ultrafine TiO_2 aerosols (21 nm P25 particles) by inhalation (Nurkiewicz, et al., 2008). Pulmonary instillation of SWCNT has been shown to produce vascoconstrictive and prothrombotic effects in rats (Schladweier, et al., 2009).

III. TEST RECOMMENDATION

Inhalation is the preferred method of exposure of the respiratory tract for hazard identification and to obtain dose-response data for use in quantitative risk assessment. A 90-day inhalation study by nose-only administration in male or female rats with up to 3 months observation post exposure is recommended for evaluating the toxicity and carcinogenicity potential of [redacted] ([redacted] Since bronchoalveolar lavage fluid (BALF) analysis can provide sensitive biochemical markers for toxic responses and information on early signs of cytotoxicity, inflammation and fibrosis (Henderson et al., 1985) -- important mechanistic information for particle toxicity/carcinogenicity, it is recommended to include BALF analysis in the 90-day inhalation study. Therefore, there are several additional considerations for using the 90-day subchronic protocol at CFR [redacted]

i. Evaluation includes markers of damage, oxidant stress, cell proliferation, the degree/intensity and duration of pulmonary inflammation, fibrosis, and cytotoxic effects in the broncoalveolar lavage fluid (BALF) and histopathology of pulmonary and ex-pulmonary organs/tissues (cardiovascular, CNS, liver, kidney, etc.,). It is also recommended to determine the potential for cardiovascular toxicity through monitoring of the most sensitive blood and/or plasma endpoints indicative of cardiovascular effects.
ii. Data on pulmonary deposition (lung burden), clearance half-life (biopersistence~~)~~ and translocation of the test material are desirable to aid in interpreting the test results.
iii. Differences in the physicochemical properties of the as-administered material – relative to the as-produced material -- should be provided (to include size distribution information in iii, and other toxicologically relevant properties). Techniques used to produce the as-administered material should also be described.
iv. The proposed protocol for testing should be provided to EPA for review and comment prior to the initiation of testing.
v. The results of the subchronic inhalation study may support the need to conduct a 2-year inhalation study bioassay in rats with the PMN.

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Appendix B

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

08/04/08

MEMORANDUM

SUBJECT: [redacted] Immunotoxicity Standard Review

FROM: Ronald E. Ward Ph.D., Microbiologist/Immunologist
RAD/SSB (7403M)

THRU: Donald Rodier, Branch Chief
RAD/SSB (7403M)

TO: James Kwiat, Technical Integrator
NCSAB (7403M)

TSCA [redacted]

Conclusion: To the extent that [redacted] (Nano-scale carbon based product consisting of open-ended rolls of graphite carbon sheets) is respirable to the deep lung, this PMN would be an immunotoxicity hazard. Exposure to this PMN compound would therefore have the potential to cause adverse effects to the lung, including inflammation, cell damage, reduced bacterial clearance, and systemic immunotoxicity. In addition, the metal impurities may cause asthma.

Basis for Conclusion: [redacted] will be used as a filler in thermoplastic polymers for antistatic applications [redacted]), a filler for thermosetting polymers for antistatic applications ([redacted]), and a filler in metals for mechanical enforcement ([redacted]) (1, 2). This PMN is a solid with a MW of [redacted] The submitters also report that the PMN has manganese, cobalt, aluminum oxide, and magnesium oxide as impurities.

The PMN was tested negative for skin sensitization via the Guinea Pig Maximization Test (3). Therefore, this PMN would not be a skin sensitization hazard.

There were no other immunotoxicity data associated with this PMN. Therefore, the conclusions reached here are based on analog published scientific data.

Some of the PMN particles may be respirable to the deep alveolar regions of the lung. In general, inhalation of particulates may lead to reduced alveolar macrophage phagocytosis and impaired clearance of inhaled bacteria (4). This reduced clearance may arise from direct toxicity to the cell, from cell overloading, or from functional inhibition with mediators such as prostaglandin E2.

Inhalation of particulates may also lead to pulmonary inflammation. Such inflammation may damage cells of the lung and may lead to the exacerbation of asthma (5).

To the extent that [redacted] has any nano-sized particles (<100nm), carbon nanotube inhalation can also cause systemic (6) and pulmonary (7) immunotoxicity.

Two of the impurities, cobalt and aluminum, are known immunotoxicants. Cobalt is a sensitizing metal and can cause occupational asthma and other types of adverse effects to the lung (8). Some aluminum compounds also can cause occupational asthma and other pulmonary effects (9). More research is needed concerning the pulmonary immunotoxic effects due to environmental exposure to aluminum compounds (9) .

Collectively, these results support the idea that [redacted] would be an immunotoxicity hazard, if there is exposure to the deep lung. Exposure to this PMN compound has the potential to cause adverse effects to the lung, including inflammation, cell damage, reduced bacterial clearance, and systemic immunotoxicity. In addition, the metal impurities may cause asthma.

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Appendix C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

August 07, 2015

THIS MEMORANDUM CONTAINS
TSCA [redacted]

MEMORANDUM

SUBJECT: **Human Health Tests evaluation for P-15-0487-491:**

1) Acute oral toxicity study in rats
2) Acute dermal toxicity study in rats
3) Acute inhalation toxicity study in rats
4) 28-day inhalation toxicity study in rats
5) 90-day inhalation toxicity study in rats
6) In vitro genetic mutation study in bacteria
7) In vivo bone marrow erythrocyte micronucleus test in mice
8) In vitro mammalian chromosome aberration

FROM: Viktor Morozov, Ph.D.
Assessment Branch 3
Risk Assessment Division (7403M)
(Reviewed by SRC with RAD Quality Assurance)

TO: James Alwood, Program Manager
New Chemicals Management Branch
Chemical Control Division (7405M)

THRU: Louis Scarano, Ph.D., Branch Chief
Assessment Branch 1
Risk Assessment Division (7403M)

DATA EVALUATION RECORD

Submitter name: Daewoo International USA Corp.
Chemical identity:

[redacted]

P-15-0487-0491

Multi-walled carbon nanotubes; Trade name: K-Nanos-100P Grade, K-Nanos-100T Grade; Purity: >90% for all studies.

Executive Summary:

In an acute oral toxicity study, a group of three female Sprague-Dawley (SD) rats were administered P-15-0487 (purity: >90%) in DPPC solution via gavage at 300 mg/kg-bw and observed for 14 days. No mortality or clinical signs of toxicity were observed within 6 days in the first group of rats, so a second group of three female rats received the same dose and were observed for 14 days. No mortality or clinical signs of toxicity were noted. Body weight gain was normal in all animals throughout the study period. Necropsy revealed no macroscopic abnormalities. The acute oral LD50 was > 300 mg/kg-bw in female rats.

In an acute dermal toxicity study, Sprague-Dawley CD rats (5/sex/group) were dermally exposed to a single dose of P-15-0487 (purity: > 90%) at 0 or 2000 mg/kg-bw and observed for 14 days. P-15-0487, moistened with DPPC solution, was applied to the clipped, intact skin of each rat and held in place for 24 hours under semi-occlusive dressing. Following the exposure period, the dressings were removed and the test site was washed with sterile distilled water. No mortalities occurred and no signs of toxicity or skin irritation were noted during the study. No treatment-related effects on body weight were observed. Necropsy revealed no macroscopic abnormalities. The acute dermal LD50 was > 2000 mg/kg-bw in male and female rats.

In an acute inhalation toxicity study, Fisher 344 rats (5/sex/group) were exposed whole-body to P-15-0487 (purity: > 90%) for 6 hours at measured concentrations of 0 (filtered fresh air), 0.00017, 0.00052, and 0.00083 mg/L and were observed for 14 days post-exposure. No treatment-related mortality or signs of toxicity were observed during the study. There were no significant effects on body weight. No

treatment-related effects were observed at necropsy. The 6-hour LC50 was > 0.00083 mg/L in male and female rats.

In a 28-day repeated-dose inhalation toxicity study, Fischer 344 rats (10/sex/group) were exposed, nose-only, to P-15-0487 (purity: > 90%) at measured concentrations of 0, 0.00017, 0.00051, and 0.00097 mg/L for 6 hours/day, 5 days/week, for 28 days. No mortalities or PMN substance-related adverse effects were observed. The NOAEC for male and female rats was 0.00097 mg/L.

In a 90-day repeated-dose toxicity inhalation study, Fischer 344 rats (10/sex/group) were exposed, nose-only, to P-15-0487 (purity: > 90%) at measured concentrations of 0 (filtered fresh air), 0.00017, 0.00051, and 0.00101 mg/L for 6 hours/day, 5 days/week, for 13 weeks. Additional male rats (5/group) were included in the control, low-, mid-, and high-concentration groups for the assessment of recovery after a 13-week non-exposure period. No PMN substance-related mortalities or adverse effects were observed. The NOAEC for male and female rats was 0.00101 mg/L.

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed to P-15-0487 (purity: > 90%) in DPPC solution at concentrations ranging from 31 to 500 µg/plate, with and without metabolic activation. Vehicle and positive controls were included and responded appropriately. No evidence of cytotoxicity was observed. No information was provided regarding test substance precipitation. No increase in revertants was observed at any concentration with or without metabolic activation.

In an in vivo micronucleus assay, ICR mice (6 males/group) were exposed to P-15-0487 (purity: 90%) in DPPC solution at concentrations of 12.5, 25 or 50 mg/kg-bw. No signs of systemic toxicity or cytotoxicity were observed. Negative (vehicle) and positive controls were included and responded appropriately. The test item did not induce micronuclei in male or female mice exposed to P-15-0487 under the conditions of this study.

Chinese hamster ovary (CHO-k1) cells were exposed to P-15-0487 (purity: > 90%) in DPPC solution at concentrations ranging from 0.78 to 3.13 µg/mL, with and without metabolic activation. Negative (vehicle) and positive controls were included and responded appropriately. In a preliminary range-finding test, cytotoxicity was observed at concentrations ≥ 3.13 µg/mL in the presence and absence of metabolic activation, and test substance precipitation was observed at concentrations ≥ 6.25 µg/mL. No information regarding cytotoxicity or test substance precipitation was reported in the main study. No increase in the number of aberrant metaphases was observed at any concentration with or without metabolic activation.

Study 1: Acute oral toxicity study in rats

Title: Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method)

Ahn, K-S. 2014. Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method). Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea. Study No. GT13-00015. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
P-15-0487 in DPPC solution (5.5 mM D-(+)-glucose, 0.6 mg/mL bovine serum albumin, and 0.01 mg/kg 1,2-dipalmitoyl-sn-glycero-3-phosphocholine [DPPC] in Dulbecco's phosphate buffered saline) was administered via gavage at 300 mg/kg-bw in a volume of 30 mL/kg to two groups of three female Sprague-Dawley (SD) rats. The test substance (volume of 10 mL/kg) was administered three times on a single day with 2-3 hour intervals between each administration (total dosing volume of 30 mL/kg). The first group of rats was dosed and observed for 14 days. As no mortality or clinical signs of toxicity were observed within 6 days in the first set of rats, the second group of three female rats was dosed and observed for 14 days. After the 14-day observation period, the animals were sacrificed, and a gross pathological examination was conducted. Dose selection was determined in accordance with OECD TG 423 and the dose level of the first step (300 mg/kg-bw) was selected due to an absence of available toxicity information on the test substance. Justification for the choice of vehicle was based on Kim et al., 2011 [1] which showed the test substance was equally dispersed up to 1% in DPPC solution. No statistical analyses were performed. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2012-23 (2012) and OECD TG 423 (2001) and conformed to GLP standards. The study author noted that homogeneity and stability tests were not performed because the test solutions were prepared on the morning of administration. Administration of the test substance to two additional groups of rats at 2000 mg/kg-bw (the next step according to the dosing protocol in OECD TG 423) was not conducted due to the low solubility of the test substance in the vehicle (dispersion up to 1% in DPPC solution). However, these deviations were not considered to have impacted the study results or conclusions.

Results and Discussion:
The acute oral LD50 was > 300 mg/kg-bw in female rats. No mortality or clinical signs of toxicity were noted. Body weight gain was normal in all animals throughout the study period. Necropsy revealed no macroscopic abnormalities.

Conclusions:
Author's conclusions: Under the conditions of this study, the acute oral LD50 for female rats was > 300 mg/kg-bw.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions.

[1] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 2: Acute dermal toxicity study in rats

Title: Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats

Ahn, K-S. 2014. Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea. Study No. GT13-00016. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Sprague-Dawley (SD) rats (5/sex) were dermally exposed to a single dose of P-15-0487 at a dose level of 2000 mg/kg-bw and observed for 14 days. The PMN substance was applied to a 5x5 cm piece of gauze and moistened with DPPC solution (5.5 mM D-(+)-glucose, 0.6 mg/mL Bovine serum albumin, and 0.01 mg/kg 1,2-dipalmitoyl-sn-glycero-3-phosphocholine [DPPC] in Dulbecco's phosphate buffered saline). The gauze pad was placed on the clipped, intact skin (~20% of total body surface area) of each rat and held in place for 24 hours with non-irritating tape and dressing bandages. An additional group of rats (5/sex) was exposed to DPPC solution only. Following the exposure period, the dressings were removed and the test site was washed with sterile distilled water. Test animals were sacrificed at the end of the study, and a gross pathological examination was conducted. Dose selection was determined in accordance with OECD TG 402. Justification for the choice of vehicle was based on Kim et al., 2011 [2] which showed the test substance was equally dispersed up to 1% in DPPC solution. Body weights were analyzed using the Independent Samples t-Test. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-1 (2013) and OECD TG 402 (1987) and conformed to GLP standards. The study author indicated that a stability test was not performed because the vehicle solution was only used to wet the PMN substance prior to application. However, the lack of stability testing was not considered to have impacted the study results or conclusions.

Results and Discussion:
The acute dermal LD50 was > 2000 mg/kg-bw in male and female rats. No mortalities occurred. No signs of toxicity or skin irritation were noted during the study. Average body weights were slightly decreased (1-4%) in all animals of both the vehicle control and treatment groups one day following administration, as compared to body weights prior to administration. There were no statistically significant differences in body weight between the vehicle control and treatment groups. All animals gained body weight throughout the remainder of the observation period. Necropsy revealed no macroscopic abnormalities.
Conclusions:
Author's conclusions: Under the conditions of this study, the acute dermal LD50 was estimated to be > 2000 mg/kg-bw in male and female rats.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions.

[2] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 3: Acute inhalation toxicity study in rats

Title: Acute Inhalation Toxicity Study of MWCNT in Fischer 344 Rats

Choi, B-G. 2014a. Acute Inhalation Toxicity Study of MWCNT in Fischer 344 Rats. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea. Study No. GT13-00173. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fischer 344 rats (5/sex/group) were exposed whole-body to P-15-0487 for 6 hours at target concentrations of 0 (filtered fresh air), 0.0002, 0.0005, and 0.001 mg/L (measured concentrations of 0, 0.00017, 0.00052, and 0.00083 mg/L, respectively) and observed for 14 days. The highest exposure concentration was the maximal mass concentration capacity for the carbon nanotube generating system; low- and mid-concentrations were determined by the standard high-concentration dilution process. The geometric mean cumulative median length (±SD) of the PMN substance was 233.97±1.57 nm. Body weights and results of lung function tests were analyzed by one way analysis of variance and Dunnett's test. Statistical analyses were conducted with a minimum significance level of 5%. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-02 (2013) and OECD TG 403 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The 6-hour LC50 was > 0.00083 mg/L. No treatment-related mortality or signs of toxicity were observed during the study. There were no significant effects on body weights. No treatment-related effects were observed at necropsy.

Conclusions:
Author's conclusions: The 6-hour LC50 was > 0.00083 mg/L in male and female rats.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 4: 28-day inhalation toxicity study in rats

Title: Subacute Inhalation Toxicity Study of MWCNT in Fischer 344

Choi, B-G. 2014b. Subacute Inhalation Toxicity Study of MWCNT in Fischer 344. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea. Study No. GT13-00174. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; black powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fisher 344 rats (10/sex/group) were exposed nose-only to P-15-0487 for 6 hours/day, 5 days/week, for 28 consecutive days at target concentrations of 0 (filtered fresh air), 0.0002, 0.0005, or 0.001 mg/L (analytically measured concentrations: 0, 0.00017, 0.00051, and 0.00097 mg/L, respectively). Target exposure concentrations were selected based on the results of an acute inhalation toxicity study in rats (Choi, 2014a, reviewed herein) in which no mortality or toxic signs were observed up to 0.00083 mg/L, the highest concentration tested. The highest exposure concentration was the maximal mass concentration capacity for the carbon nanotube generating system. The geometric mean cumulative median length (±SD) of the PMN substance was 395.33±1.51 nm. Continuous data were analyzed using the standard one-way analysis of variance and Duncan's or Dunnett's test. Non-continuous data were analyzed by Chi-squared analysis. Statistical analyses were conducted with a significance level of $p < 0.05$. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-2 (2013) and OECD TG 412 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The NOAEC for male and female rats (determined by the reviewer) is 0.00097 mg/L, based on no adverse exposure-related effects observed at the highest concentration tested. A LOAEC could not be determined.

No mortalities occurred. There were no PMN substance-related effects on clinical observations, body weight, body weight change, food consumption, hematology, urinalysis, organ weights, gross findings, or histopathological findings. Food consumption of high-concentration males was significantly decreased by 15%, compared with controls, during week 1. Food consumption of high-concentration females was significantly increased by 11% during week 4. The changes in food consumption were not considered to be treatment-related as body weight and body weight change were not affected. Significantly increased (13%) magnesium levels were observed in males' blood of the high-concentration group. There was a concentration-related trend for increased magnesium levels as an increase of 8% was observed in males of the mid-concentration group. Potassium levels were also significantly increased (7%) in males of the high-concentration group. Plasma glucose levels were decreased in a concentration-dependent manner in male rats from the mid- and high-concentration groups (11% and 13%) when compared with the control group. Plasma glucose levels for males of the high-concentration group were statistically significantly different from control levels. Albumin/globulin ratio was significantly decreased by 5% in male rats from the low- and high-concentration groups when compared to the control. The study author stated that the clinical chemistry changes were not treatment-related as the values either fell within the normal physiological range and/or the changes were not statistically concentration-dependent. No data demonstrating the normal physiological range for clinical chemistry were included

in the study report. However, the biological relevance of the clinical chemistry changes is unknown as there were no correlating changes in organ weights or histopathology. Histopathological changes were observed at a similar incidence in the control and high-concentration animals and were not considered to be PMN substance-related.

Conclusions:
Author's conclusions: Exposure to the test substance for 28 days did not have any significant health effects on the rats in this study.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion. The study author did not determine LOAEC and NOAEC values in the study. The reviewer considers the NOAEC for male and female rats to be 0.00097 mg/L, as no adverse effects were observed at the highest concentration tested. The LOAEC could not be established. It is noted that the highest concentration tested did not result in toxic effects, but the highest concentration was the maximal mass concentration capacity for the carbon nanotube generating system.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 5: 90-day inhalation toxicity study in rats

Title: Subchronic Inhalation Toxicity Study of MWCNT in Fischer 344

Choi, B-G. 2014c. Subchronic Inhalation Toxicity Study of MWCNT in Fischer 344. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea. Study No. GT14-00042. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; black powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fisher 344 rats (10/sex/group) were exposed nose-only to P-15-0487 for 6 hours/day, 5 days/week, for 13 weeks at target concentrations of 0 (filtered fresh air), 0.0002, 0.0005, or 0.001 mg/L (analytically measured concentrations: 0, 0.00017, 0.00051, and 0.00101 mg/L, respectively). Additional male rats (5/group) were included at all concentrations for the assessment of recovery after a 13-week non-exposure period. Target exposure concentrations were selected based on the results of an acute inhalation toxicity study in rats and a 28-day repeated-dose inhalation study in rats (Choi, 2014a, b; reviewed herein) in which no mortality or toxic signs were observed at the highest concentrations tested, 0.00083 mg/L and 0.00097 mg/L, respectively. The highest exposure concentration for this study was the maximal mass concentration capacity for the carbon nanotube generating system. The geometric mean cumulative median length (±SD) of the PMN substance was 566.54±1.88 nm. Continuous data were analyzed using the standard one-way analysis of variance and Duncan's or Dunnett's test. Non-continuous data were analyzed by Chi-squared analysis. Statistical analyses were conducted with a significance level of $p < 0.05$. The study, which was conducted in 2014, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-2 (2013) and OECD TG 413 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The NOAEC for male and female rats is 0.00101 mg/L, based on no adverse treatment-related effects observed at the highest concentration tested. A LOAEC could not be determined.

One animal in the mid-concentration group died on study day 81 after exhibiting restlessness, convulsions, and stupor on study day 80. The animal had red exudate in the abdominal cavity; inflammation, flattening of the uroepithelium, lumen dilatation, and red urine in urinary bladder; pulmonary and hepatic congestion; focal mineralization of the renal tubule; and prostate edema, hemorrhage, and inflammation. The death was not considered to be related to exposure.

No other mortalities or clinical signs were observed in the main study or during the recovery period. In the main study, there were no PMN substance-related effects on body weight, food consumption, ophthalmoscopy, hematology and blood coagulating parameters, female urinalysis parameters, female absolute organ weights, relative organ weights, gross necropsy findings, bronchoalveolar lavage test, or microscopic findings. During the recovery period, there were no differences in body weight, food consumption, ophthalmoscopy, hematology parameters, urinalysis parameters, absolute and relative organ weights, gross necropsy findings, bronchoalveolar lavage test, or microscopic findings among the groups.

In female rats of the main study, significantly increased ($p<0.05$) sodium levels (2%) were observed at all concentrations and significantly increased potassium (6%) levels were observed at the mid- and high-concentrations, compared with controls. During the recovery period, prothrombin time was significantly ($p<0.05$) increased by 10 and 9%, in males of the mid- and high-concentration groups, respectively. In males of the low- and high-concentration recovery groups, cholesterol levels were significantly increased by 8 and 7%, respectively, and magnesium levels were significantly decreased by 16 and 13%, respectively. In the main study, there was a statistically significant increase ($p<0.05$) in trace urine ketone bodies in males of the mid- and high-concentration groups; incidence of 0/5, 1/5, 3/5, and 5/5 at control, low-, mid-, and high-concentration, respectively. The incidence of urine ketone bodies (grade 1+) was 5/5, 4/5, 1/5, and 0/5 at control, low-, mid-, and high-concentration, respectively, showing a concentration-related decreasing trend with statistical significance in the mid- and high-concentration groups. A concentration-related trend for increasing urine pH values in PMN substance-exposed males was also observed. The findings were of uncertain toxicological relevance due to lack of gross and histological findings in the kidney. A statistically significant increase (18%) in absolute left lung weight was observed in males of the high-concentration group. The study author stated that the significant blood coagulation, clinical chemistry, and organ weight changes were not treatment-related as the values either fell within the normal physiological range and/or the changes were not concentration-dependent. No data demonstrating the normal physiological range were included in the study report. However, the biological relevance of the blood coagulation, clinical chemistry, and urine ketone body and pH changes is unknown as there were no correlating changes in organ weights or histopathology. Histopathological changes were observed in one or two animals or at a similar incidence in the control and exposed males and were not considered to be PMN substance-related.

Conclusions:
Author's conclusions: The NOAEC for male and female rats is 0.00101 mg/L, and a target organ was not identified.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion that the NOAEC for male and female rats is 0.00101 mg/L, based on no adverse treatment-related effects observed at the highest concentration tested; the LOAEC could not be determined. It is noted that the highest concentration tested did not result in toxic effects, but the highest concentration was the maximal mass concentration capacity for the carbon nanotube generating system. It is also noted that no females were included in the recovery group, and justification was not provided.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 6: In vitro genetic mutation study in bacteria

Title: Bacterial Reverse Mutation Test of MWCNT

Kim, J.S. 2014. Bacterial Reverse Mutation Test of MWCNT. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories (Korea). Laboratory Study No. GT13-00017. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
A GLP-compliant bacterial reverse mutation assay with Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA was conducted using the preincubation method. This study complied with OECD TG 471 (1997) and National Institute of Environment Research (NIER) Notice No. 2012-23 (revised August 22, 2012). The strains were supplied by Molecular Toxicology Incorporated. In the preliminary range-finding test and main study, TA98, TA100, TA1535, TA1537, and WP2uvrA were exposed to the PMN substance in the vehicle (DPPC solution [5.5 mM D-(+)-glucose + 0.6 mg/ml Bovine serum albumin + 0.01 mg/kg 1,2-dipalmitoyl-sn-glycero-3-phosphocholine [DPPC] in Dulbecco's phosphate buffered saline]) at 0, 31, 63, 125, 250, or 500 µg/plate, with and without metabolic activation. The rationale for the selection of the concentrations was not reported. Both tests were conducted in triplicate. The positive controls in the absence of metabolic activation were the following: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA98, TA100, and WP2uvrA), sodium azide (TA1535), and 9-aminoacridine hydrochloride hydrate (TA1537). In the presence of metabolic activation, 2-aminoanthracene was the positive control for all strains. All positive controls were dissolved in dimethyl sulfoxide. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence and absence of metabolic activation were calculated. Justification for the choice of vehicle was based on Kim et al., 2011 [3] which showed the test substance was equally dispersed up to 1% in DPPC solution. No deviations from the study protocol were noted.

Results and Discussion:
No increase in revertants was observed with or without metabolic activation in the main study. No evidence of cytotoxicity was observed in the range-finding test and main study. No information was provided regarding test substance precipitation. The vehicle and positive control data were within or close to the ranges established by the laboratory's historical data.

Conclusions:
Author's conclusions: Under the conditions of this study, the test substance did not induce gene mutations.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions that the test substance is negative for mutagenicity under the conditions of the study.

[3] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

The reviewer notes the following study deficiencies or deviations from OECD TG 471: the test item was not tested at the recommended maximum concentration of 5 mg/plate; the number of cells per bacterial culture was not provided; 2-aminoanthracene was used as the sole indicator of the efficacy of the S9-mix; and no historical control data were provided although the authors did provide ranges of the acceptable number of revertants for vehicle and positive controls.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 7: In vivo bone marrow erythrocyte micronucleus test in mice

Title: Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR Mice

Kim, J.S. 2014. Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories (Korea). Laboratory Study No. GT13-00019. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
A GLP-compliant in vivo bone marrow erythrocyte micronucleus test was conducted using ICR mice. This study complied with OECD TG 474 (1997) and National Institute of Environment Research (NIER) Notice No. 2012-23 (revised August 22, 2013). In the main test, ICR (CrljOri: CD1) mice (6 males/group) were administered the PMN substance in the vehicle (DPPC solution [5.5 mM D-(+)-glucose + 0.6 mg/ml Bovine serum albumin + 0.01 mg/kg 1,2-dipalmitoyl-sn-glycero-3-phosphocholine [DPPC] in Dulbecco's phosphate buffered saline]) at 0, 12.5, 25, or 50 mg/kg-bw. The maximum dose was chosen to be 50 mg/kg-bw because no mortalities were observed at this dose in a preliminary range-finding test. A positive control group comprised of 6 male mice received 2.0 mg/kg-bw mytomycin-C. Bone marrow was harvested at 24 hours after PMN substance administration, and at least 2000 erythrocytes were evaluated per animal by microscopic examination for the number of micronucleated polychromatic erythrocytes (MNPCE). At least 200 erythrocytes were evaluated per animal to determine the numbers of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). The ANOVA test was used to compare the micronuclei frequency and PCE/(PCE+NCE) ratios of the negative control and treated groups. In the event that statistical significance was established by the ANOVA test, linear logistic regression was used to test for dose-response. Additionally, the ANOVA test was used to evaluate the body weights of test animals at necropsy, and Dunnett T3 or Duncan's multiple range test was used if statistical significance was established. Justification for the choice of vehicle was based on Kim et al., 2011 [4] which showed the test substance was equally dispersed up to 1% in DPPC solution. No deviations from the study protocol were noted.

Results and Discussion:
The PMN substance did not induce micronuclei in bone marrow erythrocytes in male mice. Administration of the PMN substance did not result in a decrease in the PCE/(PCE+NCE) ratio, indicating that cytotoxicity was not observed. No clinical signs or statistically significant changes in body weight were noted in the test groups compared to the negative control group. The positive control induced a statistically significant ($p < 0.05$) increase in the frequency of MNPCEs and a statistically significant ($p < 0.05$) decrease in the PCE/(PCE+NCE) ratio.

Conclusions:
Author's conclusions: The test item did not induce micronuclei in the bone marrow erythrocytes of the ICR mouse.

[4] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

Reviewer's conclusions: Based on the reported results, micronuclei were not induced in mice bone marrow. Due to numerous deficiencies in reporting, as well as deviations from OECD TG 474, the reviewer considers the results of this study to be inconclusive.

The reviewer notes the following study deficiencies or deviations from OECD TG 474: the highest dose tested did not cause toxicity in the main study and a sufficient justification was not provided for the selection of doses; no justification was provided for only testing male animals; samples of bone marrow were collected only once instead of twice within 48 hours; the same treatment regimen may not have been used in the preliminary study (oral administration) and main study (oral or i.p. injection- unclear in report); rationale for route of administration was not provided; inconsistent reporting of doses used in the preliminary test [500, 1000, and 2000 mg/kg-bw (p. 10 of study report) vs. 12.5, 25, and 50 mg/kg-bw (p. 6 of study report)]; inconsistent reporting of methods [e.g., test substance dispersed in distilled water (p. 10 of study report) vs dispersed in DPPC solution (p. 4 of study report); negative control group received corn oil (p. 6 of study report) vs. DPPC solution (Tables 1-2, pp. 12-13)]; and no methods were used to verify that the test substance reached the target tissue.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 8: In vitro mammalian chromosome aberration

Title: In vitro Mammalian Chromosome Aberration Test of MWCNT Using Cultured Chinese Hamster Ovary (CHO-k1) Cells

Kim, J.S.. 2014. In vitro Mammalian Chromosome Aberration Test of MWCNT Using Cultured Chinese Hamster Ovary (CHO-k1) Cells. Performing Laboratory: Bioconvergence Technology Laboratory, Korea Conformity Laboratories (Korea). Laboratory Study No. GT13-00018. Sponsor: Bioconvergence Technology Laboratory, Korea Conformity Laboratories, Korea.

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
A GLP-compliant in vitro mammalian chromosome aberration test was conducted with Chinese hamster ovary (CHO-k1) cells. This study complied with OECD TG 473 (1997) and National Institute of Environment Research (NIER) Notice No. 2012-23 (revised August 22, 2012). The CHO-k1 cells were obtained from Korean Cell Line Bank. In the preliminary range-finding test, CHO-k1 cells were exposed to the PMN substance in the vehicle (DPPC solution [5.5 mM D-(+)-glucose + 0.6 mg/ml Bovine serum albumin + 0.01 mg/kg 1,2-dipalmitoyl-sn-glycero-3-phosphocholine [DPPC] in Dulbecco's phosphate buffered saline]) at 0 (vehicle control), 1.56, 3.13, 6.25, 12.5, 25, 50, 100 or 200 µg/mL in the absence of metabolic activation for 6 or 24 hours, or in the presence of metabolic activation for 6 hours. Based on the results of the range-finding experiment, in the main test, CHO-k1 cells were exposed to the PMN substance in the vehicle at 0 (vehicle control), 0.78, 1.56 or 3.13 µg/mL in the absence of metabolic activation for 6 or 24 hours, or in the presence of metabolic activation for 6 hours. Duplicate cultures were tested at each concentration for each experimental condition. All cells were harvested at 24 hours. The positive control in the absence of metabolic activation was mitomycin C. The positive control in the presence of metabolic activation was cyclophosphamide. The percentage of aberrant metaphases, excluding gaps, and frequency of cells with polyploidy or endoreduplication at each concentration were calculated for each experimental condition. Statistical significance, as compared to the vehicle control, was determined using a Chi-squared test. A linear regression test was performed for dose-response. Justification for the choice of vehicle was based on Kim et al., 2011 [5] which showed the test substance was equally dispersed up to 1% in DPPC solution. No deviations from the study protocol were noted.

Results and Discussion:
In the range-finding test, cytotoxicity (>50% decrease in relative cell count, defined by the reviewer) was observed at concentrations ≥ 3.13 µg/mL and precipitation was observed at ≥ 6.25 µg/mL, with and without metabolic activation. In the main test, the test substance did not induce a statistically significant increase in the frequency of chromosome aberrations in CHO-k1 cells exposed for 6 or 24 hours without metabolic activation or 6 hours with metabolic activation. Additionally, the test substance did not induce a statistically significant increase in the frequency of polyploidy or endoreduplication under any treatment condition. No information was provided regarding observations of cytotoxicity or precipitation in the main test. In the negative (vehicle) control group, the frequency of

[5] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

chromosome aberrations ranged from 0-0.5. The positive controls induced statistically significant ($p < 0.05$) increases in the frequency of chromosome aberrations under all test conditions.

Conclusions:
Author's conclusions: Under the conditions of this study, the test substance did not induce chromosome aberrations in Chinese hamster ovary (CHO-k1) cells.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion that the test substance is negative for clastogenicity under the conditions of the study.

The following study deficiencies or deviations from OECD TG 473 were noted: cytotoxicity criteria were not clearly defined in the report, although the highest analyzable concentration, 3.13 µg/mL, caused a >50% decrease in relative cell count; no historical vehicle or positive control data were provided; and the study report did not specify the incubation temperature during testing.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.